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"Effect of Stress Factors on Nutrition"

SUMMARY

The Effects of B Vitamins, Liver and Yeast
on Atabrine Toxicity in the Rat

Immature female rats were maintained for eight weeks on purified rations containing 500 mg of atabrine per kg of diet, and the effects of feeding were contrasted with that observed on similar rations with atabrine omitted. Four experimental rations were employed: (1) a basal ration containing the B complex factors in synthetic form only (2) the basal ration plus additional B vitamins (3) the basal ration plus yeast and (4) the basal ration plus desiccated whole liver.

The effects of atabrine feeding differed significantly on the various diets employed. Administration of atabrine plus basal ration resulted in a marked retardation of growth, alopecia, inhibition of ovarian development, enlarged submaxillary glands, granulocytosis and myocardial damage as indicated by electrocardiographic tracings. These effects were largely counteracted by diets containing whole liver or yeast and to a lesser extent by the administration of additional B vitamins. On atabrine-free rations no abnormalities were observed on any of the diets employed.

Desiccated whole liver was more effective than yeast or the additional B vitamins in promoting growth and ovarian development in the immature atabrine-fed rat. The protective factor(s) was present in the water-insoluble fraction of liver remaining after removal of the extractable water-soluble material.

An increased B vitamin requirement was observed in immature rats fed toxic doses of atabrine. The suggestion is made that in addition to the known B vitamins still other factors are present in whole liver and yeast that are required in increased amounts in the atabrine-fed rat.

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Introduction

Available data indicate that in addition to the major nutrients, substances are present in our diet which may be required in increased amounts during conditions of stress. Such factors are apparently dispensable under normal conditions or their requirements are so small they may readily be met by amounts present in the diet or through the synthetic activity of the intestinal flora or the animals' own tissues. Certain drugs or other "stress factors" may, however, increase requirements for these substances to the extent that deficiencies occur, manifest by retarded growth or tissue pathology, and preventable by the administration in appropriate amounts of the missing nutrient (Ershoff, '47a). In the present communication data are present indicating that atabrine is such a stress factor. Administration of this drug in toxic amounts resulted in the young rat in retardation of growth, alopecia, inhibition of ovarian development, granulocytosis and other pathological effects preventable, at least in part, by the administration of an unknown nutrient present in liver and yeast.

Procedure and Results

Four basal rations were employed in the present experiment: diets A, B, C and D. Diets A and B were purified rations containing the B complex factors in synthetic form only. Diets C and D were similar in composition but contained yeast or desiccated whole liver in addition to the synthetic vitamins. Sixty-four female rats of the Long-Evans strain were selected at 21 to 23 days of age and an average weight of 41.8 gm. for the present experiment. Animals were kept in metal cages with raised screen bottoms to prevent access to feces, and were fed ad libitum the diets listed in Table I. Feeding was continued for 8 weeks or until death, which ever occurred sooner. During the eighth week electrocardiograms were taken of all rats. Animals were autopsied on the 56th day of feeding; ovarian and submaxillary weights were determined; and sections of the ovaries, submaxillary glands, liver and heart prepared for microscopic study. Tissues were fixed in 10% formol and stained with hematoxylin and eosin.

Table I
(see end of paper)

Findings indicate that the toxic effects of atabrine in the immature rat may be counteracted by dietary means. Pathological effects of atabrine were most pronounced on the synthetic ration, diet A, and least evident on the liver-containing diet D. Atabrine-fed rats on diet A exhibited a marked retardation of growth (table II), extensive alopecia, infantile ovaries and enlarged submaxillary glands (table II), granulocytosis (table III) and electrocardiographic abnormalities. Three of the 10 rats on this diet failed to survive the experimental period of 8 weeks; and of the remaining 7 animals only 2 gained weight after the second week of feeding. Eight rats in this series developed extensive alopecia during the first month of feeding although new hair had generally replaced the areas of alopecia by the eighth week. At autopsy ovaries appeared infantile both in weight and microscopic appearance. The most striking finding, however, was a marked hypertrophy of the submaxillary glands. These weighed 3 to 4 times more than those observed in normal animals of simialr weight and were significantly heavier than those observed in the much larger atabrine-free controls (table II). Histologically the increased size of the submaxillary glands appeared to be due almost entirely to hyperplasia and hypertrophy of mucous cells.[1]

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1 We are indebted to Professor E.M. Hall, Dept. of Pathology, University of Southern California Medical School, for the examination of the histological material.

Retardation of growth was similarly observed in atabrine-fed rats on diets B, C and D although growth was significantly greater on these rations than on diet A. Atabrine-fed rats gained most weight on the liver-containing diet D with growth somewhat less on diets containing yeast (diet C) or the additional B vitamins (diet B.) Three rats on the latter ration plateaued in weight after the first few weeks of feeding; the remainder of the group, however, and all atabrine-fed rats on diets C and D gained weight consistently during the experimental period. With the exception of the 3 rats on diet B that plateaued in weight, alopecia was not observed in any of the atabrine-fed rats except those on diet A.

Ovaries appeared infantile both in weight and microscopic appearance in all atabrine-fed rats on diet A. They were somewhat larger in atabrine-fed rats on diets B and C but were still significantly smaller than those observed in atabrine-free controls. On diet D no significant difference in ovarian weight was observed between animals fed atabrine and those on similar rations with atabrine omitted; and histologically the ovaries of atabrine-fed rats in this group appeared normal in all respects. On rations free of atabrine ovarian weights did not differ significantly on any of the diets employed; and histologically ovaries appeared normal in all groups (table II.)

Enlargement of the submaxillary glands was directly correlated with alopecia and failure to gain weight. Enlarged submaxillaries were only observed in atabrine-fed rats that lost fur and plateaued in weight (8 rats on diet A; 3 on diet B), with glands apparently normal both in size and histological appearance in remaining animals of the atabrine series. In no instance were submaxillary glands enlarged on atabrine-free rations (table II).

Table II
(see end of paper)

Per cent and total granulocytes per cc. of blood were markedly increased in atabrine-fed rats on diet A. During the sixth week of feeding total and differential white cell counts, hemoglobin determinations and total red cell counts were made on the tail blood of all surviving rats. Differential counts were made on smears stained with Wright's stain, 100 cells on each of 2 slides being employed for each analysis. All blood counts were made in duplicate.

No significant difference in total erythrocytes or hemoglobin levels was observed on the various diets tested between animals fed atabrine and those on atabrine-free rations. Erythrocytes averaged 7.3 to 8.1 million per cc. of blood for the various groups (range 6.4 - 9.3 million per cc.), with hemoglobin averaging 15.4 to 16.1 mg./100 cc. (range 14.6 - 17.2 mg./100 cc.). Total leucocytes per cc. of blood did not differ significantly on the various rations tested; a significant increase in per cent and total granulocytes per cc. of blood was observed, however, in atabrine-fed rats on diet A. Such was not the case with atabrine-fed rats on other diets tested nor for animals on atabrine-free rations (table III).

Table III
(see end of paper)

In agreement with earlier findings (Hegsted, McKibbin and Stare, '44) no consistent abnormalities were observed histologically in the liver and myocardium of atabrine-fed rats on the various diets employed, nor did these tissues differ significantly from those of animals fed similar rations with atabrine omitted[1]. These findings are in contrast to those of Wright and Lillie ('43) and Siegel and Mushett ('44) who observed necrosis and replacement fibrosis in the liver and myocardium of atabrine-fed rats. These differences may be due, at least in part, to the amount of atabrine fed, the composition of the diets employed or a strain difference in response to atabrine feeding.

Myocardial damage in atabrine-fed rats was indicated, however, by means of electrocardiographic tracings. Electrocardiograms were taken with a Cambridge-Hindle 2 galvanometer electrocardiograph (research type unit) of all surviving rats during the eighth week of feeding. Resistance was standardized for each animal, and the standard three leads were taken on unanesthetized rats at a paper speed of 100 cm. per second[2]. A marked elevation of the ST segment (indicative of myocardial damage) was observed in the electrocardiographic tracings of 5 of the 7 atabrine-fed rats on diet A and 5 of the 9 atabrine-fed rats on diet B.[3] Elevated ST segments did not occur in tracings obtained from rats fed diets C_2 or D_2 or from animals fed atabrine-free rations. Further abnormalities in the atabrine series consisted of a prolonged PR interval (indicative of delayed AV conduction) in animals fed diet D_2. Six of the 10 rats on the latter ration had a PR interval of 0.05 seconds or longer in contrast to an approximate value of 0.04 seconds in virtually all rats fed other rations tested. With the exception of the above, electrocardiograms of atabrine-fed rats did not differ significantly from controls either in complexes or in ventricular rate[4]. No abnormalities were observed in the electrocardiograms of atabrine-fed rats on diet C.

In subsequent work experiments were conducted in an attempt to concentrate the factor or factors in liver responsible for its protective effect. Immature female rats of the Long-Evans strain were weaned at 21 to 23 days of age and fed ad libitum the following 3 diets: (1) diet A (2) diet A plus Liver Concentrate Powder 1-20 (Wilson) added at a level of 4% of the ration and (3) diet A plus Extracted Liver Residue (Wilson) added at a level of 10%. The liver fractions were added in place of an equal amount of sucrose[5]. All diets were supplemented with 500 mg. of atabrine per kg. of diet. Feeding was continued for 8 weeks (10 animals per group.)

Findings indicate the protective factor or factors is either water-insoluble

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2 We wish to express our sincere appreciation to Dr. John C. Ruddock, for the examination and description of the electrocardiographic tracings. Dr. Ruddock is Clinical Professor of Medicine, University of Southern California Medical School, Chief of Medical Service and Head of the Department of Cardiology, St. Vincent's Hospital, Los Angeles, California.

3 ST segments were elevated on all 3 leads on diet B_2 and on leads II and III on diet A_2. Slight elevations of the ST segment were occasionally noted in leads II and III on all control rations and on diets C_2 and D_2. In no case, however, were they so pronounced as those on diets A_2 or B_2.

4 Heart rates averaged 504 to 537 per minute for all groups with an individual range of 465 to 570 per minute. The QRS interval averaged 0.02 seconds for all groups.

5 We are indebted to Dr. David Klein of the Wilson Laboratories, Chicago, Ill., for the Liver Concentrate Powder 1-20 and the Extracted Liver Residue employed in the present experiment.

or is chemically bound so that it may not be readily removed by simple water extraction. No significant difference in growth or gross appearance was observed between atabrine-fed rats on diet A and those receiving Liver Concentrate Powder 1-20 (containing the water-extractable material of raw liver.) On the other hand, Extracted Liver Residue (consisting of the coagulated, water-insoluble material remaining after the removal of the extractable water-soluble substances) was virtually as effective as whole liver powder in counteracting atabrine toxicity in the rat. Animals fed the above rations gained the following amounts of weight during the 8 week feeding period; diet A, 41.6 ± 10.4 gms; diet A plus Liver Concentrate Powder, 52.8 ± 9.7 gms; diet A plus extracted Liver Residue, 92.7± 9.3 gms.

Discussion

Available data indicate that factors are present in liver and yeast that will counteract, at least in part, the effects of drug toxicity in the rat. As early as 1922 Funk ('22) expressed the view that the composition of the diet and perhaps its vitamin content may have a profound influence on the toxicity of drugs. This suggestion has been amply confirmed, not only in regard to the known nutrients (Ershoff, '47a) but to additional factors present in liver or yeast. The beneficial effects of the latter in animals inhaling carbon tetrachloride or fed toxic doses of strychnine, promin, dinitrophenol, sulfanilamide and other drugs has been recognized for years (De Santibanez, '47; Battelli,'40; Higgins,'44; Chamelin and Funk, '43). Similar results have been observed following toxic doses of diethyl-stilbestrol (Funk and Funk,'39; Chamelin and Funk,'43), estrogen (Engel and Rosenberg,'45; Ershoff,'47b,c). The present experiment indicates that atabrine toxicity may also be counteracted, at least in part, by the administration of whole liver or yeast.

Findings indicate that the effects of atabrine administration in the immature female rat are dependent on the diet employed. With rats fed a synthetic ration (diet A), administration of atabrine at a level of 500 mg per kg of diet resulted in marked retardation of growth, alopecia, inhibition of ovarian development, enlarged submaxillary glands, granulocytosis and myocardial damage as indicated by electrocardiographic tracings. The above effects were largely counteracted by the administration of desiccated whole liver at a level of 10% of the diet in place of an equal amount of sucrose. On this latter ration (diet D_2) growth was significantly greater than on diet A_2; alopecia did not occur; ovaries and submaxillary glands appeared normal both in weight and microscopic appearance; granulocyte counts were normal; and electrocardiograms were free of the abnormalities observed on diet A_2 although some prolongation of the PR interval was observed. Similar results were obtained with diet C_2 (in which yeast was fed in place of the whole liver) although growth was less than on diet D_2 and ovaries resembled in weight and microscopic appearance those of an immature rat. Electrocardiograms in this series, however, remained free of the abnormalities observed on other atabrine-containing rations and were indistinguishable from those of normal controls. Findings on diet B_2 were intermediate between those on diet A_2 and rations containing whole liver and yeast. Three of the 10 rats on this diet failed to grow; they developed alopecia and at autopsy revealed markedly enlarged submaxillary glands. The remainder of the series, however, were indistinguishable grossly or in microscopic appearance from animals fed yeast (diet C_2). Electrocardiographic tracings revealed a marked elevation of the ST segment similar to that observed on diet A_2 in 5 out of the 9 rats on this ration. On atabrine-free diets no abnormalities were observed on any of the rations employed.

Since the toxic effects of atabrine were less pronounced on diet B_2 than on diet A_2 and since these two diets differed only in their content of known B vitamins, it is apparent that the beneficial effects of diet B_2 were due to its increased content of B vitamins. The latter ration contained twice the thiamine, riboflavin, pyridoxine, pantothenate and niacin content of diet A as well as significant amounts of biotin, folic acid, inositol and p-aminobenzoic acid. It would appear that one or more of the latter factors were responsible for the observed effects. The toxic effects of atabrine were still, however, more pronounced on diet B_2 than on yeast or whole liver-containing rations (diet C_2 or D_2). These findings would indicate that in addition to the known B vitamins there were additional factors in the whole liver and yeast that counteracted, at least in part, the effects of atabrine toxicity in the rat. Preliminary results indicate the protective factor(s) is present in the water-insoluble fraction of liver remaining after the removal of the extractable water-soluble material.

References

Battelli, G. 1940 Detoxicating action of liver extracts in experimental strychnine poisoning. Boll. soc. ital. biol. sper., vol. 15, p. 687; Chem. Abstr. 1946 vol. 40, 58339

Chamelin, I.M. and C. Funk 1943 The action of liver extracts in counteracting the toxic effects of diethyl-stilbestrol and sulfanilamide. Arch. Biochem., vol. 2, p. 9

De Santibanez, J.D. 1943 The detoxifying principle in liver, Rev. facultad. cienc. quim. vol. 18, p. 177; Chem Abstr. 1947, vol. 41, 1040i

Engel, P. and E. Rosenberg 1945 Estrogen-inactivation liver extracts. Endocrinology vol. 37, p. 44

Ershoff, B.H. 1947a Conditioning factors in nutritional disease. Physiol. Rev., in press

Ershoff, B.H. 1947b Effects of liver feeding on growth and ovarian development in the hyperthyroid rat. Proc. Soc. Exp. Biol. and Med., vol. 64, p. 500

Ershoff, B.H. 1947c Comparative effects of liver and yeast on growth and length of survival of the immature thyroid-fed rat. Arch. Biochem., in press

Ershoff, B.H. and H.J. Deuel, Jr. 1946 The beneficial effects of yeast on the body and gonadal weight of immature rats fed alpha-estradiol. Amer.J.Physiol., vol. 145, p. 465

Ershoff, B.H. and D.Hershberg 1945 The beneficial effects of yeast on the cardiac failure of hyperthyroid rats. Amer. J. Phyiol., vol. 145 p. 16

Funk, C. 1922 Die Vitamie, II Ed., Bergmann, Wiebaden, p. 341

Funk, C. and I.C. Funk 1939 The action of certain hormones as dietary constituents. Science, vol. 90, p. 443

Hegsted, D.M., J.M. McKibbin, and F.J. Stare 1944 Nutrition and tolerance to atabrine. J. Nutrition, vol. 27, p. 141

Higgins, G.M. 1944 The influence of purified diets upon the toxicity of promin. Am. J. Clin. Path., vol. 14, p. 278

Siegel, H. and C.W. Mushett 1944 Structural Changes following administration of quinacrine hydrochloride. Arch. Path., vol. 38, p. 63

Sure, B. 1941 Dietary requirements for fertility and lactation. XXIX. The existence of a new dietary factor essential for lactation. J. Nutrition, vol. 22, p. 499

Wright, C.I. and R.D. Lillie 1943 Toxic effects of atabrine and sulfadiazine in the growing rat. Public Health Rep., vol. 58, p. 1242

Table 1

Composition of Experimental Diets

Dietary component	Diet A_1 and A_2	Diet B_1 and B_2	Diet C_1 and C_2	Diet D_1 and D_2
Yeast[1]	0.0	0.0	10.0	0.0
Whole Liver Powder[2]	0.0	0.0	0.0	10.0
Vitamin Test Casein[3]	22.0	22.0	22.0	22.0
Salt Mixture[4]	4.5	4.5	4.5	4.5
Sucrose	73.5	73.5	63.5	63.5

Atabrine (quinacrine hydrochloride)[5] was incorporated in diets A_2, B_2, C_2 and D_2 at a level of 500 mg. per kg. of diet, replacing an equal amount of sucrose.

To each kg. of diet A, C and D were added the following synthetic vitamins: thiamine hydrochloride 72 mg., riboflavin 9 mg., pyridoxine hydrochloride 15 mg., calcium pantothenate 67.2 mg., nicotinic acid 60 mg., 2-methyl-naphthoquinone 5 mg., and choline chloride 1.2 gm.

To each kg. of diet B were added thiamine hydrochloride 144 mg., riboflavin 18 mg., pyridoxine hydrochloride 30 mg., calcium pantothenate 134.4 mg., nicotinic acid 120 mg., inositol 1.2 gm., p-aminobenzoic acid 600 mg., folic acid 10 mg., biotin 1 mg., 2-methyl-naphthoquinone 10 mg, and choline chloride 1.2 gm.

Each rat also received three times weekly the following supplement; cottonseed oil (Wesson) 500 mg., alpha-tocopherol 1 mg., and a vitamin A-D concentrate[6] containing 50 U.S.P. units of vitamin A and 5 U.S.P. units of vitamin D.

Footnotes to Table 1.

1 Brewer's Type Yeast No. 200, Anheuser-Busch, Inc., St. Louis, Mo.

2 Whole Dried Liver Powder, Armour and Co., Chicago, Ill.

3 Vitamin Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio

4 Salt Mixture No. 1 (Sure, '41).

5 Quinacrine HCl Powder (Atabrine), Winthrop Chemical Co., New York, N.Y.

6 Nopco Fish Oil Concentrate, assaying 800,000 U.S.P. units of vitamin A and 80,000 U.S.P. units of vitamin D per gram.

Table II

Effects of atabrine on growth and ovarian and submaxillary weight in the immature female rat

Dietary group	Number of animals	Initial body weight	Gain in body wt. over 8 wk. period	Average ovarian wt.[1]	Average submaxillary wt.[1]	Ratio of submaxillary wt. to body wt. 10^{-3}
		gm.	gm.	mg.	mg.	
Atabrine Series						
A	10	41.7	33.9 ± 12.1 (7)	12.4 ± 3.4	486.4 ± 41.6	6.43
B	10	41.9	87.4 ± 10.8 (9)	26.0 ± 3.5	418.6 ± 39.4	3.24
C	10	41.8	99.1 ± 5.1 (10)	30.4 ± 2.7	303.8 ± 10.8	2.15
D	10	41.8	118.2 ± 5.2 (10)	43.5 ± 2.9	317.8 ± 19.4	1.92
Atabrine-free Controls						
A	6	41.7	146.4 ± 9.0 (6)	47.0 ± 2.4	374.8 ± 18.4	1.98
B	6	42.0	152.3 ± 7.8 (6)	49.1 ± 3.0	334.6 ± 16.8	1.72
C	6	41.5	146.8 ± 6.9 (6)	43.2 ± 3.2	317.3 ± 32.8	1.69
D	6	41.7	169.7 ± 9.3 (6)	44.8 ± 2.1	313.5 ± 14.8	1.49

The values in parentheses indicate the number of animals which survived and on which averages are based.

Footnotes to Table II.

1 Including standard error of the mean calculated as follows:

$\sqrt{\frac{\Sigma d^2}{n}} \Big/ \sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations.

Table III

Effects of atabrine on the granulocyte count of the rat.

Dietary group	Number of animals	Total leucocyte count			Granulocytes	
		Average[1]		Range	Per cent[1]	Total[1] per cc.
Atabrine Series						
A	9	17,090	1,540	10,200 - 24,800	43.9 ± 4.3	7503 ± 735
B	10	15,900	1,310	8,600 - 24,100	23.0 ± 1.7	3657 ± 270
C	10	13,280	1,650	9,200 - 20,400	24.3 ± 2.5	3227 ± 332
D	10	12,270	1,280	7,900 - 18,400	21.5 ± 2.3	2638 ± 282
Atabrine-free controls						
A	6	13,150	860	8,800 - 15,800	18.6 ± 2.6	2446 ± 342
B	6	12,870	1,040	7,800 - 18,100	17.4 ± 2.8	2239 ± 360
C	6	14,180	810	8,200 - 16,600	19.1 ± 1.6	2708 ± 227

Footnotes to Table III.

1 Including standard error of the mean calculated as follows:

$$\sqrt{\varepsilon \frac{d^2}{n}} \Big/ \sqrt{n}$$

where "d" is the deviation from the mean and "n" is the number of observations.